AD_____

Award Number: **W81XWH-12-1-0094**

TITLE: **Identification of Cell Nonautonomous DNA Damage Responses in the Tumor Microenvironment that Contribute to Cancer Therapy Resistance**

PRINCIPAL INVESTIGATOR: **Ryan R. Gordon, Ph.D.**

CONTRACTING ORGANIZATION: **Fred Hutchinson Cancer Research Center**
Seattle, WA 98109-1024

REPORT DATE: **May 2014**

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
May 2014	Annual Summary	28 February 2012 – 27 February 2014

4. TITLE AND SUBTITLE
Identification of Cell Nonautonomous DNA Damage Responses in the Tumor Microenvironment that Contribute to Cancer Therapy Resistance

5a. CONTRACT NUMBER
W81XWH-12-1-0094

5b. GRANT NUMBER
W81XWH-12-1-0094

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Ryan R. Gordon, Ph.D.

E-Mail: rrgordon@fhcrc.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fred Hutchinson Cancer Research Center
P.O. Box 19024, J6-500
Seattle, WA 98109-1024

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
A major impediment to effective prostate cancer treatment involves the acquired resistance to cytotoxic therapies. Components of the tissue microenvironment are increasingly recognized to profoundly influence tumor cell phenotypes that include susceptibilities to toxic insults. Using a genome-wide analysis of transcriptional responses to genotoxic stress induced by cancer therapeutics, we have identified a spectrum of secreted proteins derived from the tumor microenvironment (TME) that have the *potential* to modify tumor growth and enhance resistance to DNA-damaging cancer therapeutics. These results suggest a mechanism by which genotoxic therapies given in a cyclical fashion can enhance subsequent treatment resistance through cell non-autonomous effects contributed by the TME. To date, the contributions of individual members of this DNA Damage-associated Secretory Program (DDSP) have not been defined, nor have the signaling mechanisms responsible for propagating the DNA-damage signal(s) been determined. Our objective during this grant period is to test whether treatment-associated DNA damage responses in cells comprising the prostate TME promote tumor growth and subsequent therapy resistance. During this funding period we have: (1) Generated a prostate fibroblast cell line stably expressing SPINK1; (2) Evaluated the impact which SPINK1 activation has upon the growth characteristics of prostate cancer cells lines; (3) Examined how SPINK1 secretion from the microenvironment modulates the response of prostate cancer cells to chemotherapeutics: (4) Begun the evaluation of SPINK1 regulatory pathway; (5) Evaluated the impact of SPINK1 overexpression on tumor growth *in-vivo.*

15. SUBJECT TERMS
Prostate cancer, Microenvironment, DNA Damage-associated Secretory Program, SPINK1

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	16	

Table of Contents

Introduction

A major impediment to effective prostate cancer treatment involves the acquired resistance to cytotoxic therapies. Components of the tissue microenvironment are increasingly recognized to profoundly influence tumor cell phenotypes that include susceptibilities to toxic insults. Using a genome-wide analysis of transcriptional responses to genotoxic stress induced by cancer therapeutics, we have identified a spectrum of secreted proteins derived from the tumor microenvironment (TME) that have the *potential* to modify tumor growth and enhance resistance to DNA-damaging cancer therapeutics. These results suggest a mechanism by which genotoxic therapies given in a cyclical fashion can enhance subsequent treatment resistance through cell non-autonomous effects contributed by the TME. To date, the contributions of individual members of this DNA Damage-associated Secretory Program (DDSP) have not been defined, nor have the signaling mechanisms responsible for propagating the DNA-damage signal(s) been determined. The research supported by this award aims to test whether treatment-associated DNA damage responses in cells comprising the prostate TME promote tumor growth and subsequent therapy resistance. Our specific aims are as follows: (1) Determine the contribution of specific effectors of the tumor microenvironment-derived DDSP in modulating resistance to cytotoxic chemotherapy and ionizing radiation; (2) Determine the mechanism(s) by which the DDSP is activated; (3) Determine if therapeutic targeting of SPINK1 in the TME can attenuate therapy resistance.

Body

The following summarizes the research accomplishments throughout the duration of the proposal award period, as associated with each task in the Statement of Work.

Task 1: Determine the contribution of specific effectors of the tumor microenvironment-derived DDSP (e.g. SPINK1) in modulating resistance to cytotoxic chemotherapy and ionizing radiation. (Months 1-10).

We choose to focus on SPINK1 for this task based on the following reasons: First, we found SPINK1 to be highly induced (~20-fold) in the TME following chemotherapy (1); Second, SPINK1 is secreted and was recently shown to engage the EGFR pathway and promote cell proliferation (2); Third, antibodies to SPINK1 have been shown to be effective in blocking SPINK1 activity, thus providing a clear translational pathway (2); Fourth, while SPINK1 is overexpressed by a minority (about 10% of prostate cancers) (3), our data indicate that SPINK1 may be a highly relevant target in most prostate cancer patients that undergo treatment with genotoxic agents such as radiation or chemotherapy.

Task 1a: Generation of stable PSC27 cell lines both overexpressing SPINK1 and with SPINK1 silenced. Complete confirmation experiments of altered SPINK1 expression using western blot, quantitative PCR and immunofluorescence. (Months 1-3).

Lenti-viral particles containing ORF constructs coding for constitutively active SPINK1 and a GFP-marker were generated and used to transduce PSC27 immortalized prostate fibroblasts cells. Cells were then maintained under the selective pressure of Blasticidin S for several weeks in order to eliminate non-transduced cells resulting in a population stably overexpressing SPINK1 transcript. In parallel PSC27 cells were similarly targeted with lenti-viral particles containing shRNA's targeting SPINK1 transcriptional activity. shRNA targeted cells were then maintained under Puromycin selective pressure until the population uniformly

expressed the GFP-tag. In addition empty-vector PSC27 cell lines for both the overexpression and silenced constructs were generated for use as controls in future experiments. Transcript abundance for all lines was verified using quantitative PCR and protein abundance via western blot (Figure 1). Our results revealed that we were able to stably induce SPINK1 transcript production to levels consistent with those found in prostate fibroblasts following γ-irradiation. Further, we were able to verify that protein was secreted into the conditioned medium. This stable overexpression and secretion was critical for our downstream experiments.

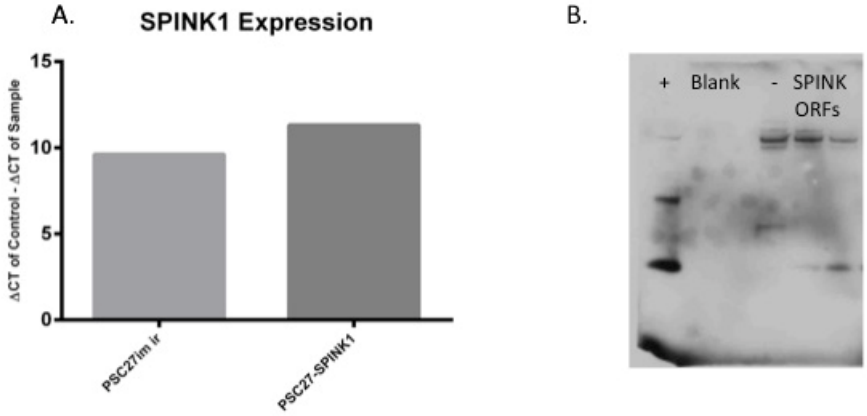

Figure 1. Verification of induced SPINK1 transcript and protein. A: The change in transcript of SPINK1 compared to untreated control cells for both irradiated PSC27im and PSC27im cells transduced with the ORF construct. B: The lower bands on the western blot represent SPINK1 protein detected in the conditioned medium collected from the SPINK1 ORF cell lines along with protein detected in our positive control of pancreatic lysate.

Task 1b: Obtain institutional approval for all proposed animal experiments. (Month 1-4).

All approvals are now in place.

Task 1c: Complete co-culture experiments with prostate cancer cell lines (PC3, DU145, VCaP, 22rv1) and the PSC27^{SPINK1} fibroblasts. Evaluate epithelial cell growth compared to control PSC27 fibroblasts. (Months 3-5).

This task was designed to mimic the growth response of prostate cancer cells when exposed to a SPINK1 activated environment. It further allows us to evaluate how microenvironment signaling can contribute to the growth rates of a primary tumor. To evaluate this response several prostate cancer cell lines were grown with or without presence of overexpressed SPINK1 derived from prostate fibroblast cells. Following 48 hours of treatment total cell numbers were quantitated and compared against controls. These results revealed that SPINK1 overexpression enhanced cell growth in all of the prostate cancer lines evaluated (Figure 2).

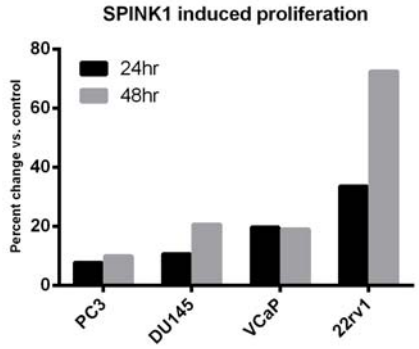

Figure 2. A SPINK1 enriched environment induced increased proliferative activity amongst prostate cancer cell lines. Each cell line was grown in a SPINK1 enriched environment for either 24hrs or 48hrs alongside a matching control. After the prescribed time-period the percent change vs. the control was measured via a MTS assay.

Task 1d: Generation of conditioned media from stable PSC27 cell lines overexpressing SPINK1 and with SPINK1 silenced. (Months 3-5)

We are continually generating fresh conditioned media for each experiment. The media is prepared by adding serum free DMEM to a flask of cells which is ~70% confluent and allowing them to grow for three days. After the prescribe time has elapsed the media is collected and sterile filtered prior to use. The presence of SPINK1 in the conditioned media was verified using western blot analysis and activity confirmed by measuring the activation of EGFR in treated cancer cell lines.

Task 1e: Complete treatments with conditioned media with on prostate cancer cell lines (PC3, Du145, 22rv1, LNCaP, VCaP). Expose cells to IC50 concentrations of chemotherapeutics and/or ionizing radiation. Following exposure to the cytotoxic therapies quantitate growth characteristics and apoptosis. These studies were expanded to include invasion assays. (Months 5-14).

Prostate cancer cells were exposed to IC50 concentrations of docetaxel and supplemented with conditioned media generated from the overexpressing SPINK1 cell line or the matched control. Cells were allowed to grow for 48hrs in these conditions prior to evaluating growth characteristics and apoptosis. Our results indicate that there were varying effects of SPINK1 on the growth of prostate cells when exposed to docetaxel (Figure 3). Specifically, both PC3 and Du145 cells had a slight increase in cell growth compared to the matched control while had a modest reduction in cell growth when exposed to CM and docetaxel. No change in cell growth was observed for either 22rv1 or LNCaP cells when compared to controls of the same conditions. When apoptosis was evaluated we found that conditioned media supplemented Du145 and Vcap cells had similar level of apoptosis compared to controls when exposed to docetaxel (Figure 4). In the remaining cell lines we observed a reduction in apoptosis when supplemented with conditioned medium suggesting that SPINK1 may provide some level of protection to cytotoxic therapies (Figure 4).

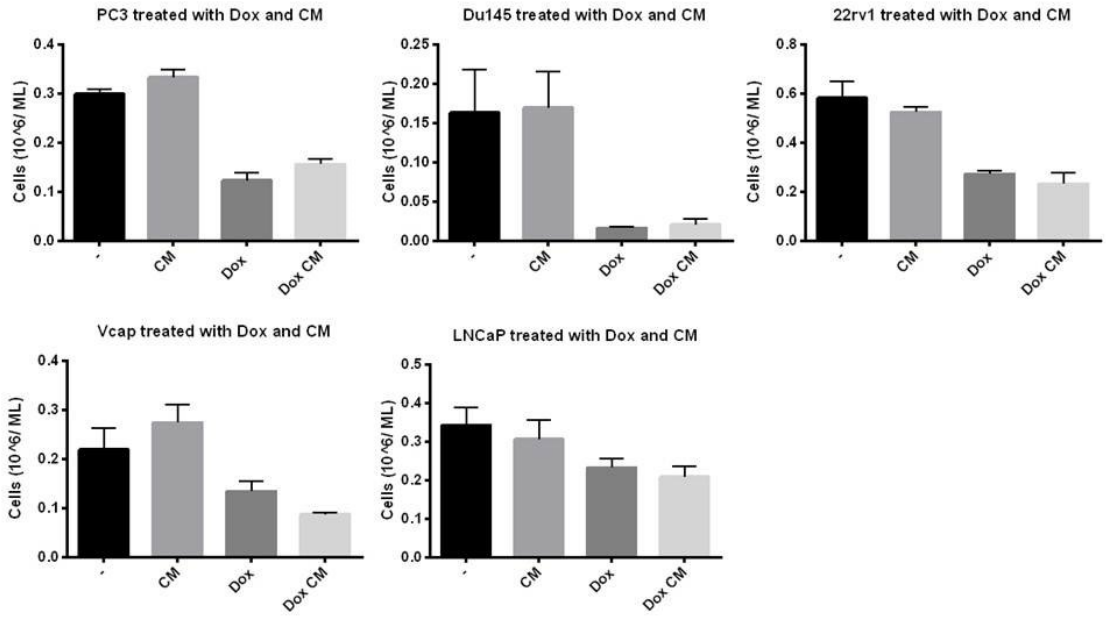

Figure 3. Growth response of prostate cancer cell lines when exposed to docetaxel (Dox) and SPINK1 conditioned medium (CM) for 48 hours. Enhanced cellular growth was observed for PC3 and Du145 cells after Dox treatment when supplement with CM. Surprisingly a reduction in cell growth was detected after Dox treatment with CM supplementation for Vcap cells. While no change in growth rates were observed after treatment for 22rv1 or LNCaP cells.

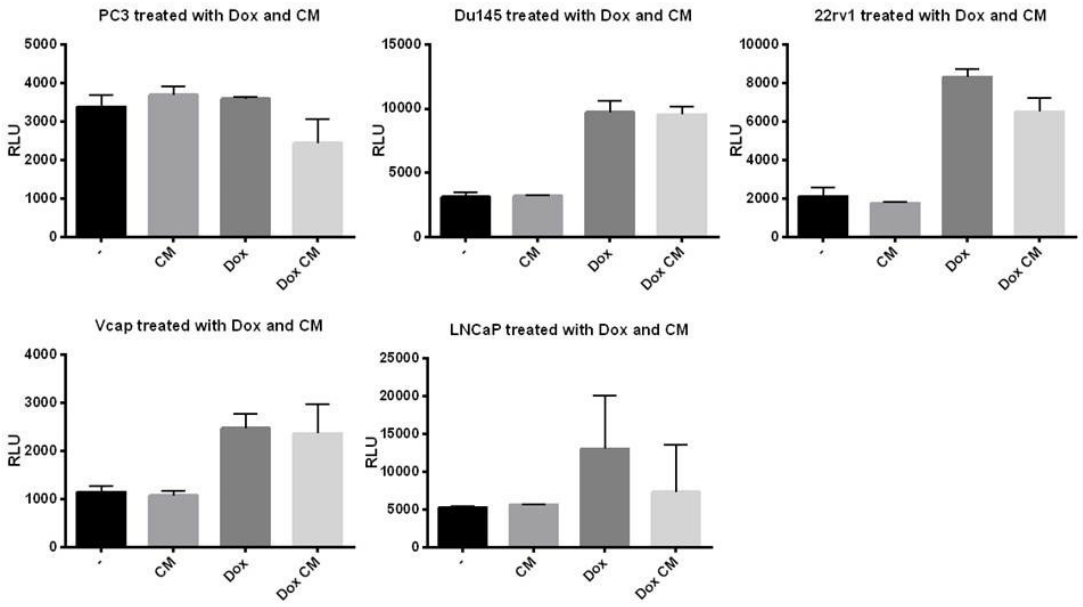

Figure 4. Apoptosis response of prostate cancer cell lines when exposed to docetaxel (Dox) and SPINK1 conditioned medium (CM) for 48 hours. No change in apoptosis was observed for Du145 and Vcap cells after Dox treatment when supplement with CM. In the remaining cell lines a reduction in apoptosis was detected after Dox treatment with CM supplementation compared to the matched controls.

To measure the effect of SPINK1 supplementation on cell invasion, cells were cultured in collagen coated boyden chambers above a well containing a chemo-attractant for 48hrs at which point invasion was quantified. Our results indicate that there were varying effects of SPINK1 on the invasive capabilites of prostate cancer cells (Figure 5). Specifically, both PC3 and LNCaP cells exhibited increased invasion compared to the matched control. No change in invasion was observed for either 22rv1 when compared to controls of the same conditions. A modest reduction in invasion was observed in Du145 cells

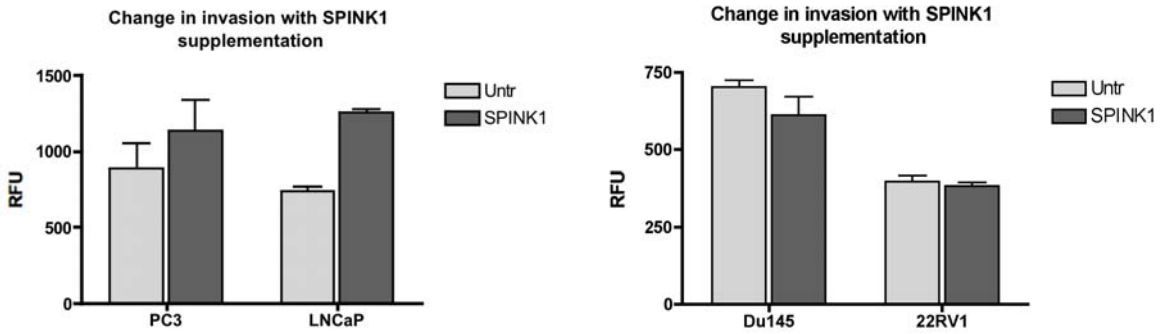

Figure 5. Measure of invasion for prostate cancer cell lines when exposed to SPINK1 conditioned medium (CM) for 48 hours. No change in was observed for 22RV1 cell when supplement with CM, while a modest reduction in invasion was observed in Du145 cells. In the remaining cell lines increased invasion was detected after CM supplementation compared to the matched controls.

***Task 1f*:** Xenograft implantation of 3 study arms using 10 SCID mice per arm. Arm 1 will combine prostate cancer cells with the PSC27^{SPINK1} fibroblasts and implant the recombinants

under the renal capsule of immune-deficient recipient mice. Arm 2 will combine prostate cancer cells with the silenced PSC27^{SPINK1} fibroblasts. Arm 3 will use only prostate cancer cells. (Months 16-20).

To assess the response to SPINK1 supplementation in an *in vivo* setting CB57-SCID mice were implanted with a combination of LNCaP cells and either empty vector control or SPINK1 overexpression cells. Each animal received a subcutaneous injection in the left flank containing 500,000 of each cell type plus matrigel. LNCAP cells were chosen because they are EGFR positive and responsive to SPINK1 CM. Animals were allowed to recover until a tumor burden of 400 mm^3 was reached, at which point tumors were measured multiple times per week. Once tumors had grown for 4 weeks post enrollment animals were sacrificed, tumors were collected and gross autopsies were performed.

Subtask 1: Harvest grafts after 4 weeks and compare growth against controls. Perform statistical analysis of outcomes.

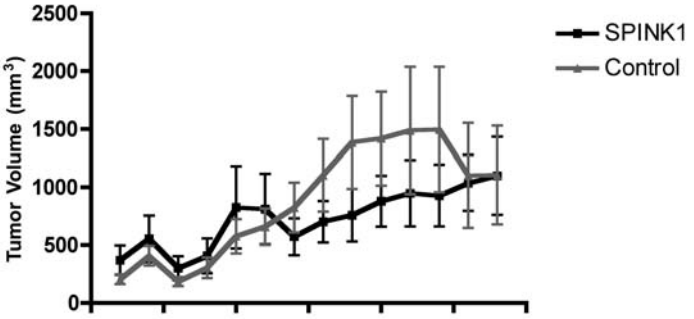

Figure 6. In-vivo tumor growth measurements. Animals were subcutaneously implanted with LNCaP cells and either control or SPINK1 overexpression cells. Tumor burden was measured several times a week over the course of four weeks. No significant change was change in was observed between the two treatment groups throughout the duration of the study.

Subtask 2: Assess the histologic appearance of the grafts by H&E staining and evaluate the tissue architecture.

No differences were observed between the treatment groups.

<u>Task 2</u>: Determine the mechanism(s) by which the DDSP is activated. (Months 8-18).

Task 2a: Generate PSC27 prostate fibroblasts cell line with stable expression of a dominant negative NFkB inhibitor (mutant IκBα). (Months 8-10).

The purpose of this task was to investigate the contribution that NFκB signaling has in the activation of SPINK1 signaling. NFkB was targeted given the presence of NFkB binding sites in the promoter region SPINK1. To accomplish this task we generated a PSC27 fibroblast cell line that stably expressed a dominant negative NFkB inhibitor (PSC27$^{I\kappa B\alpha}$). These cells could then be exposed to cytotoxic therapies and compared against control cell lines to establish the importance of NFkB in this signaling pathway.

***Task 2b*:** Expose the PSC27$^{I\kappa B\alpha}$ cells to DNA damage, through ionizing radiation, and quantitate SPINK1 expression. Perform statistical analysis of outcomes. (Months 10-12).

The PSC27$^{I\kappa B\alpha}$ and controls cell lines were exposed to 10 gray of ionizing radiation and then allowed to incubate for 12 days at 37°C. This time point was chosen after previous experiments indicated that 12 days post irradiation corresponded to peak SPINK1 transcript levels. After the prescribed period of time cells were collected and SPINK1 transcript abundance was measured. Our results revealed that SPINK1 transcript was unregulated following ionizing radiation in both the control and PSC27$^{I\kappa B\alpha}$ cell lines (Figure 7). If NFkB signaling were solely responsible for SPINK1 activation we would expect transcript levels to remain unchanged following the treatment. However, we did detect a slight increase in transcript levels for the PSC27$^{I\kappa B\alpha}$ cells after radiation therapy suggesting that NFkB, while involved, is only one component of the SPINK1 regulatory pathway.

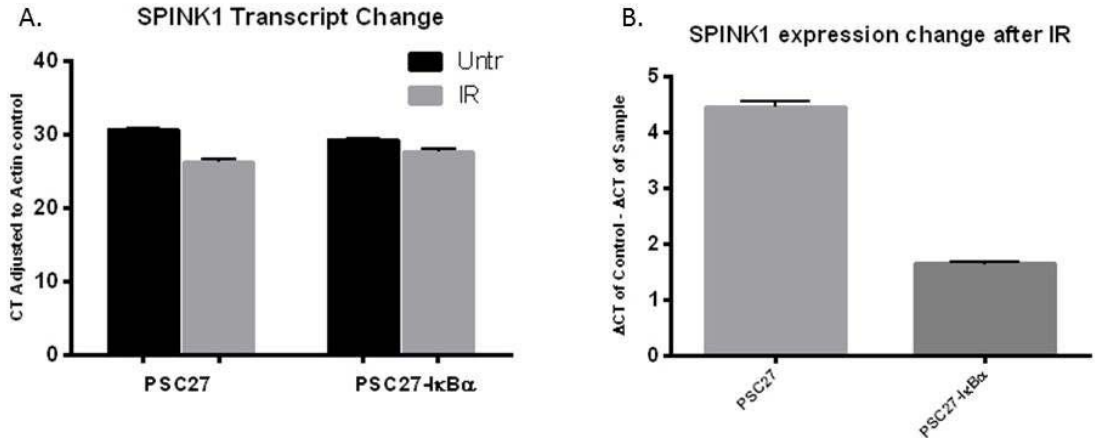

Figure 7. The Quantitative PCR measurement of SPINK1 transcript change in the PSC27$^{I\kappa B\alpha}$ cell line following exposure to 10 gray of ionizing radiation (IR). A: SPINK1 transcript abundance was measured 12 days post IR for both controls and PSC27$^{I\kappa B\alpha}$ cell lines. A smaller CT count indicates increased transcript abundance. B: The difference in CT cycles between the control and PSC27$^{I\kappa B\alpha}$ cell lines pre and post IR. The positive changes indicate an increased abundance of transcript following IR for both cell lines.

Task 2c: Complete the evaluation of NFkB binding to the SPINK1 promoter using a ChIP assay detecting chromatin captured by NFkB antibodies and PCR primers specific to sequences flanking SPINK1 promoter regions before and after ionizing radiation. Perform statistical analysis of outcomes. (Months 12-14).

Following irradiation cells were incubated for 12 additional days at which point they were formalin fixed and collected. During collection only a small amount of cell material was recovered. The poor yield was attributed to the extended incubation following damaging treatment and a shift in the cells to a senescent phenotype. As a result of the poor yield these studies were postponed in favor of the luciferase assay described below.

Task 2d: Complete the functional assessment of the NFkB binding sites in the promoter region of SPINK1. (Months 13-18).

In order to further evaluate the regulatory pathway of SPINK1 signaling we generated full length and partial sequences of the promoter region. These sequences we designed to encompass different binding site regions of the SPINK1 promoter and therefore be used for our downstream analyses.

Subtask 1: Generate SPINK1 luciferase reporter vectors comprising different regions of the SPINK1 promoter and transfect them into PSC27 cells. (Months 13-16).

To evaluate transcription factor binding that results in the activation of SPINK1 the above-mentioned promoter constructs were cloned into the PGL3 dual-luciferase system. This system includes the PGL3 basic vector encoded with a luciferase reporter and the PGL3 control vector encoded with the rinella reporter. The reporter constructs were simultaneously transiently transfected into PSC27im cells, which were then exposed to various SPINK1 activating treatments. The samples were then processed according the manufactures' protocol and luciferase and rinella activity was evaluated. Rinella represented transfection efficiency and luciferase was a measure of SPINK1 activation. While in most situations we were able to achieve a high efficiency for the transfection, SPINK1 activation was difficult to achieve. We are redesigned the SPINK1 promoter constructs and looking for more consistent activators of SPINK1. However, these constructs likewise failed to provide us an accurate readout of SPINK1 activation. We proceeded to alter the conditions of the transient transfections, performing the assay at different intervals both before and after irradiation but these also failed to yield useful information.

Subtask 2: Complete treatment on luciferase reporter cell lines with TNFα, a known activator of NFkB signaling, and evaluate response. Perform statistical analysis of response. (Months 15-18).

Given the difficulties with the dual luciferase reporter assay this approach was cancelled. We avoided investing resources into this assay due to a lack of a reliable and reproducible system. Given that the PSC27$^{I\kappa B\alpha}$ studies demonstrated that NFkB signaling contributes to SPINK1 signaling, future studies into this paradigm are warranted.

<u>Task 3</u>: Determine if therapeutic targeting of SPINK1 in the TME can attenuate therapy resistance. (Months 15-23).

Given the lack of significant differences observed in the initial SPINK1 overexpression xenograft trials we decided to forgo the subsequent xenograft analyses based on the costs versus benefits of these studies. Instead efforts were focused on the troubleshooting of the luciferase assays and the newly added invasion assays in task *1e*.

Task 3a: Xenograft implantation study using 11 SCID mice per arm. The first arm will combine prostate epithelial tumor cells with the PSC27 fibroblasts and implant the recombinants into the left flanks of immune-deficient recipient mice. The remaining arm will combine prostate epithelial tumor cells with the silenced PSC27^{SPINK1} fibroblasts and implant the recombinants into the same site as the previous arms. (Months 15-17). Forgone due to poor preliminary results.

Subtask 1: Monitor xenograft tumors until enrollment volume of 400 mm^3 is reached. (Months 16-17).

***Task 3b*:** Complete treatment studies in xenograft mice. Mice will be exposed to systemic DNA damage and given twice-weekly doses of a mAB to SPINK1 (10 mg/kg) or a placebo and treatment effects on tumor volume will be monitored. Perform statistical analysis of outcomes. (Months 17-19). Forgone due to poor preliminary results.

***Task 3c*:** Complete the cell specific evaluation of tumors collected at the end of task 12. (Months 19-23). Forgone due to poor preliminary results.

> ***Subtask 1:*** Complete IHC and Western analyses for SPINK1 (fibroblasts), Ki67 (epithelium), EGFR (epithelium) and EGFR pathway activation (epithelium) (Months 19-20)
>
> ***Subtask 2:*** Complete the isolation of major cell types (epithelium, fibroblasts) from tumors using flow cytometry. (Months 20-21)
>
> ***Subtask 3:*** Perform RNA extraction and amplification. Quantitate components of the DDSP and perform data analysis. (Months 21-23)

Task 4: Compile data and complete drafting of manuscript for publication. (Months 20-24). Further data is required before a manuscript can be drafted.

Key Research Accomplishments

- We have successfully generated prostate fibroblasts that contain SPINK1 overexpression and control constructs. Additionally SPINK1 transcript and protein increase has been verified for these cell lines.
- Conditioned medium has been generated and secreted functionally active SPINK1 protein has been verified.
- Dual treatment of prostate cancer cell lines with conditioned medium and chemotherapeutics has been completed.
- We have evaluated the SPINK1 promoter region to determine which transcription factor binding sites are present.
- We were able to confirm that NFκB is at least partially involved in the signaling cascade regulating SPINK1 activation via use of the PSC27$^{I\kappa B\alpha}$ cells.
- The full length and partial SPINK1 promoter region has been cloned into the PGL3 dual luciferase system.
- Completed *in-vitro* co-culture assays.
- The impact of SPINK1 secretion from microenviroment on cancer cell invasion has been quantified.
- Xenograft studies measuring SPINK1 effects upon prostate cancer growth characteristics have been completed.

Reportable Outcomes

- **Gordon RR**, Sun Yu, Bergan RC, Nelson PS (2013). Identification of cell non-autonomous DNA damage responses in the tumor microenvironment that contribute to cancer therapy resistance. Abstract, 18th Annual Drug Discovery Symposium, Chicago, IL.
- Gained employment as a research assistant professor at Northwestern University, School of Medicine.

Conclusions

In summary, during the course of this proposal-funding period we have made significant progress toward our goal of understanding the role that SPINK1 plays in the DNA Damage-associated Secretory Program. We have completed the critical step of creating a functionally active stably overexpressed SPINK1 prostate fibroblast line, which serves as the foundation for many of out downstream experiments. We have also established that increased SPINK1 originating from the microenvironment can enhance the proliferative capacity of prostate cancer cells. In addition this SPINK1 rich environment can alter the response of prostate cancer cell lines to the standard of care chemotherapy, docetaxel. This may be particularly relevant finding for patients undergoing treatment for advanced prostate cancer. Further, we have established that SPINK1 up-regulation in the microenvironment has the potential to enhance the invasive properties of certain prostate cancer cell lines. We have also made headway towards understanding the regulatory pathway of SPINK1 activation. The SPINK1 promoter region has been evaluated and the transcription factor binding sites contained within have been identified. One particular transcription factor of interest (NFκB) with multiple binding sites has been further investigated via the use of a dominant negative NFkB inhibitor cell line. Irradiation of this overexpression line confirmed that NFκB is at least partially involved in the signaling cascade regulating SPINK1 activation. However, technical difficulties with the transient trasfections in to irradiated cells have prevented from fully evaluating the promoter region of SPINK1. Other approaches were pursued to complete this aspect of the proposal but these too have yet to provide the information needed to elucidate the SPINK1 regulatory mechanisms. Additionally, when SPINK1 was upregulated in an in-vivo setting no change in prostate cancer cell growth was observed. While we initially anticipated enhanced growth characteristics, this result may reflect other findings demonstrating that only a small subset of prostate cancers are impacted by elevated SPINK1 expression (3). Ultimately, as a whole our body of work has demonstrated that SPINK1 secretion from the microenviroment can be activated following DNA damaging treatments and that this has the potential to alter growth characteristics, invasiveness and response to chemotherapy of human prostate cancer cells. This information is particularly relevant given that the standard chemotherapy regimes, which utilize a cyclical pattern of on and off therapy periods to minimize damage to benign cells, may activate the DDSP and in turn contribute to treatment failure.

References

1. Sun Y, Campisi J, Higano C, Beer TM, Porter P, Coleman I, True L, Nelson PS. Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. Nat Med 2012; 18: 1359-1368

2. Ateeq B, Tomlins SA, Laxman B, Asangani IA, Cao Q, Cao X, Li Y, Wang X, Feng F, Pienta KJ, Varambally S, Chinnaiyan AM. Therapeutic Targeting of SPINK1-Positive Prostate Cancer. Sci Transl Med 2011; 3: 72ra17

3. Tomlins SA, Rhodes DR, Yu J, Varambally S, Mehra R, Perner S, Demichelis F, Helgeson BE, Laxman B, Morriss DS, Cao Q, Cao X, Andren O, Fall K, Johnson L, Wei JT, Shah RB, Al-Ahmadie H, Eastham JA, Eggener SE, Fine SW, Hotakainen K, Stenman UH, Tsodikov A, Gerald WL, Lilia H, Reuter VE, Kantoff PW, Scardino PT, Rubin MA, Biartell AS, Chinnaiyan AM. The role of SPINK1 in ETS rearrangement-negative prostate cancers. Cancer Cell 2008; 13:519-528 The role of SPINK1 in ETS rearrangement-negative prostate cancers. Cancer Cell 2008; 13:519-528

Appendices

Abstract, 18th Annual Drug Discovery Symposium, Chicago, IL.

Identification of cell non-autonomous DNA damage responses in the tumor microenvironment that contribute to cancer therapy resistance

Ryan R. Gordon[1], Yu Sun[1], Peter S. Nelson[1,2,3]
[1]Divisions of Human Biology and Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA; Departments of [2]Pathology and [3] Medicine, University of Washington, Seattle, WA

A major impediment to effective prostate cancer treatment involves the acquired resistance to cytotoxic therapies. For reasons that remain unclear, adenocarcinomas arising in the prostate appear to be particularly resistant to the cytotoxic effects of commonly used anti-neoplastic drugs. Docetaxel, the current standard of care in advanced disease, provides approximately 2 months of survival advantage, and few patients sustain durable complete remissions. Dissecting the mechanisms by which tumors develop therapy resistance is critical for understanding how cells adapt to genotoxic stress and how the efficacy of cancer treatments can be improved. Increasingly components of the tissue microenvironment (TME) are recognized to profoundly influence tumor cell phenotypes that include susceptibilities to toxic insults. Using a genome-wide analysis of transcriptional responses to genotoxic stress induced by cancer therapeutics, we previously identified a spectrum of secreted proteins derived from the TME that have the potential to modify tumor growth and enhance resistance to DNA-damaging cancer therapeutics. These results suggested a mechanism by which genotoxic therapies given in a cyclical fashion can enhance subsequent treatment resistance through cell non-autonomous effects contributed by the TME. However, to date, the contributions of individual members of this DNA Damage-associated Secretory Program (DDSP) have not been defined, nor have the signaling mechanisms responsible for propagating the DNA-damage signal(s) been determined.

We set out to determine the contribution of one highly-induced DDSP protein, SPINK1, in modulating cytotoxic resistance. Initially we verified the induction of SPINK1 from prostate fibroblast *in-vitro*. The SPINK1 DDSP response was subsequently verified *in-vivo* utilizing a set of microdissected prostate stroma samples pre/post chemotherapy exposure. To further investigate this relationship we generated a prostate fibroblast cell line overexpressing SPINK1 ($PSC27^{SPINK1}$). Prostate cancer cell lines were co-cultured with the $PSC27^{SPINK1}$ fibroblasts and growth conditions evaluated. Conditioned medium collected from the $PSC27^{SPINK1}$ cells was also utilized to treat the same prostate cancer cell lines exposed to IC50 concentrations of cytotoxic therapies. Following exposure to the cytotoxic therapies cell survival, apoptosis, and proliferation was quantitated revealing differential growth characteristics. Additionally, we are currently attempting to ascertain the mechanism(s) by which the DDSP is activated. Ultimately, this research represents an effort to define the mechanisms underlying chemotherapy resistance, which is critical both for selecting patients who may optimally benefit, and for designing new therapeutic strategies that either avoid or specifically target resistance pathways.

Updated curriculum vitae reflecting research period covered by this proposal

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Gordon, Ryan R., PhD	Research Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
California Polytechnic University, San Luis Obispo	BS	12/2002	Animal Science
University of Nebraska, Lincoln	MS	12/2005	Animal Science
University of North Carolina, Chapel Hill	PhD	11/2009	Human Nutrition
North Carolina State University, Raleigh	Postdoctoral training	08/2010	Cancer Genetics
Fred Hutchinson Cancer Research Center, Seattle	Postdoctoral training	02/2014	Cancer Biology

A. Personal Statement

I have been conducting oncology scientific research for over 10 years, with a background spanning investigations into the effect of diet on health outcomes, and the evaluation of cancer microenvironment therapy resistance. I received my PhD in Human Nutrition from the University of North Carolina at Chapel Hill in 2009, where I examined the complex genetics of metastatic mammary cancer. Specifically, during that time, I focused on elucidating the relationship between dietary fat and the genetic underpinnings of metastatic mammary cancer, and gained expertise in cancer biology/genetics, modeling of dietary by gene interactions and the identification of potential cancer therapeutic targets. After obtaining my PhD I joined the laboratory of Dr. Peter Nelson at the Fred Hutchinson Cancer Research Center, where my research focused on identifying molecular alterations in prostate cancer that contribute to the development of therapy-resistance. During my postdoctoral research studies at the FHCRC, I examined how treatment-associated DNA damage responses in cells comprising the tumor microenvironment promoted tumor growth and subsequent therapy resistance. Importantly, my work evaluated how paracrine-acting factors promoted the survival of cancer cells following cytotoxic therapies. I recently joined Northwestern University as a faculty member in the laboratory of Dr. Raymond Bergan, where I will continue my research career focusing upon drug discovery and the molecular pharmacology of novel therapeutics.

B. Positions and Honors

Positions and Employment

2010-2014 Postdoctoral Fellow, Fred Hutchinson Cancer Research Center
2014-Present Research Assistant Professor, Department of Medicine, Northwestern University

Other Experience and Professional Memberships

Active Member: American Association for Cancer Research

Previous Member: American Society of Nutrition
Previous Member: International Mammalian Genome Society

Honors

DOD Postdoctoral Fellowship recipient (2011)
2009 International Travel Award recipient, American Society of Nutrition
Graduate Student Scholar Fellowship recipient, Nutrition Research Institute (2005-2009)
International Travel Award recipient, Pathways, Networks and Systems Conference (2007)
Animal Science Graduate Student Association (ASGSA), Officer (2004)

C. Selected Peer-reviewed Publications (Selected from 13 peer-reviewed publications)

Most relevant to the current application

1. **Gordon RR**, Wu M, Huang C, Harris W, Sim H, Lucas J, Coleman I, Higano C, Gulati R, True L, Vessella R, Lange P, Garzotto M, Beer T, Nelson P (New Submission) Chemotherapy-Induced Monoamine Oxidase Expression in Prostate Carcinoma Functions as a Cytoprotective Resistance Enzyme and Associates with Clinical Outcomes. *Plos One*
2. Bluemn EG, Spencer ES, Mecham B, **Gordon RR**, Coleman I, Lewinshtein D, Mosteghel E, Zhang X, Annis J, Grandori C, Porter CR, Nelson PS (2013). PPP2R2C loss promotes castration-resistant prostate cancer growth and is associated with increased prostate cancer-specific mortality. *Mol Cancer Res,* 6: 568-578*.*
3. **Gordon RR**, Nelson PS (2012). Cellular Senescence and Cancer Chemotherapy Resistance. *Drug Resistance Updates*, 15: 121-131.
4. **Gordon RR**, La Merrill M, Hunter KW, Threadgill DW, Pomp D (2010). Dietary fat-dependent transcriptional architecture and copy number alterations associated with modifiers of mammary cancer metastasis. *Clinical and Experimental Metastasis,* 5: 279-293.
5. La Merrill M, **Gordon RR**, Hunter KW, Threadgill DW, Pomp D (2010). Dietary fat alters pulmonary metastasis of mammary cancers through cancer autonomous and non-autonomous changes in gene expression. *Clinical and Experimental Metastasis*, 2: 107-116.
6. **Gordon RR**, Hunter KW, La Merrill M, Threadgill DW, Pomp D (2008). Genotype X Diet Interactions in Mice Predisposed to Mammary Cancer: II. Tumors and Metastasis. *Mammalian Genome,* 19: 179–189.

Additional recent publications (in chronological order)

1. Johnston RB, **Gordon RR**, Spencer ES, Porter CR (2013). Internet-Based Education Materials on Active Surveillance for Prostate Cancer are not Understandable by Most Patients. Submitted *J Urology.*
2. Spencer ES, Johnston RB, **Gordon RR**, Lucas JL, Ussakli CH, Lewinshtein D, Nelson PS, Porter CR (2013). Prognostic Value of ERG Oncoprotein in Prostate Cancer Recurrence and Cause Specific Mortality. *Prostate,* 9: 905-912*.*
3. Leamy LJ, **Gordon RR**, Pomp D (2012). Epistatic control of mammary cancer susceptibility in mice may depend on the dietary environment. *Hereditary Gene,* 1:108.
4. Leamy LJ, **Gordon RR**, Pomp D (2011). Sex-, diet- and cancer-dependent epistatic effects on complex traits in mice. *Front. Gene,* 2: 71.
5. Kelly SA, Nehrenberg DL, Hua K, **Gordon RR**, Garland T, Pomp D (2010). Parent-of-origin effects on voluntary exercise levels and body composition in mice. *Physiol. Genomics,* 2: 111-120.
6. Foulds Mathes W, Nehrenberg DL, **Gordon RR**, Hua K, Garland T, Pomp D (2010). Dopaminergic Dysregulation in Mice Selectively Bred for Excessive Exercise or Obesity. *Behavioral Brain Research*, 210: 155-163.

7. **Gordon RR**, Hunter KW, La Merrill M, Threadgill DW, Pomp D (2008). Genotype X Diet Interactions in Mice Predisposed to Mammary Cancer: I. Body Weight and Fat. *Mammalian Genome,* 19: 163-178.

D. Research Support

Ongoing Research Support

NA

Completed Research Support

DoD Prostate Cancer Research Program Postdoctoral Training award (W81XWH-12-1-0094) (2012-2014)